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TISSUE ENGINEERED NANOFIBER POLY (VINYL ALCOHOL) MESH FOR THE TREATMENT OF ABDOMINAL WALL HERNIA

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ABSTRACT

Intraperitoneal positioning of the non-degradable surgical mesh may cause adhesion formation. In this work 3D absorbable poly (vinyl alcohol) (PVA) scaffold was used for tissue engineered hernia replacement which was prepared by reactive electrospinning. To determine the biocompatibility the cell affinity were studied through the adherence and toxicity tests on Human lung epithelial (A549) cell line. In vivo studies were performed on Wistar rats (n=45, 200-250 g) to evaluate the implant integration and biological respons. Abdominal wall defect was performed on the right side of the abdomen, than was covered on-lay with the PVA mesh. Each mesh was fixed by simple running suture using 4/0 polypropylene suture material. Adhesion formations were documented and measured by our modified Diamond score. Macroscopical and histological responses were performed from the samples. According to the histological examinations we found that all of the scaffolds were integrated to the host tissue and were kept their structures until the end of the long experiments. Significantly more adhesion formations were attached to the non-absorbable polypropylene suture line (n=19) than we could find on the surface of the PVA mesh (n=5). In this preliminary study demonstrated that the PVA nanofiber mesh is biocompatible and could be a promising scaffold for tissue applications.

KEYWORDS: Poly (Vinyl Alcohol), Electrospun Nanofibers, Hernia Repair, Tissue Engineering, Surgical Mesh

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Original Article

INTRODUCTION

Background

Hernia is the weakness or defect in the abdominal wall or inguinal area. Many people are affected eg. incisional hernia that occur in about 10% of patients who have abdominal surgery. One of the solutions can be the usage of surgical mesh. Several types of surgical meshes are available in regenerative medicine for hernia repair such as degradable and non-degradable types. These meshes have a specialized function in the healing process after the implantation. The complexity of the tissue engineered replacement lies its structure and porus size of the scaffold as well as the strenght of the host tissue. Polypropylene non-absorbable meshes have been used more than 50 years [1]. Intraperitoneal application of this mesh has a lot of complication, e.g. high rates of fistulas and infection, adhesion formations, mesh migration and chronic pain comparing to the preperitoneally placed mesh [2]. The ideal hernia mesh should be absorbable and biocompatible, flexible, non-adhesive, and it should degrade into nontoxic fragments. A number of processing techniques have been used to prepare polymer nanofibers. One

of the modern nanotechnological methods is the electrospinning fibre formation. Polymer based mesh has porous structure which is able to mimic the extracellular matrix and has stable molecule structure with non-toxic fragments. Poly (vinyl alcohol) is a hydrophilic polymer which is non-toxic, biocompatible and biodegradable. For the production of the PVA mesh, electrospinning technique should be used.

In the electrospinning process, a high electric field is generated between a polymer solution held by its surface tension at the end of a syringe (or a capillary tube) and a collection target. Figure 1 showed the schematic representation of electrospinning apparatus.

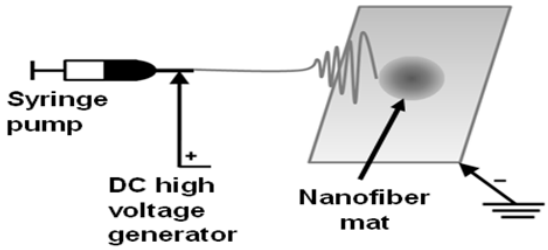

Figure 1: Electrospinning Setup

This novel biomedical mesh with good mechanical properties from high molecular weight PVA was developed by our research group [3]. PVA solution is in a capillary tube, which was fixed above a grounded tubular layer, formed a droplet due to the weight. By applied voltage, the droplet was instantly disintegrated into fibers that were drawn to the tubular layer. Applied voltage was ranged from 5 to 30 kV. The critical voltage, crossdirection width, and throughput were checked during the electrospinning process.

The purpose of this study was to test PVA nanomesh in vitro and to measure the extent of adhesion formation to PVA meshes after their intra-abdominal placement on the peritoneum, and to evaluate the host tissue response to the PVA material after the implantation.

EXPERIMENTAL METHODS

Poly (Vinyl Alcohol) and the Mesh Preparation

For the formation of the PVA mesh, electrospinning technique was used (Figure 2A). PVA was dissolved in water at 90°C for 2 h and maintained for 30 min to ensure homogenization. Concentration of PVA aqueous solution was varied from 5 to 15 wt %. The synthetized mesh showed a compact structure which thickness 1 mm. (Figure2 B-C). The mesh was sterilized in chlorine dioxid (ClO_2) solution. ClO_2 has no negative effects on the proliferation or viability of the cells although the antimitotic and antibiotic effect of the ClO_2 is well known [4].

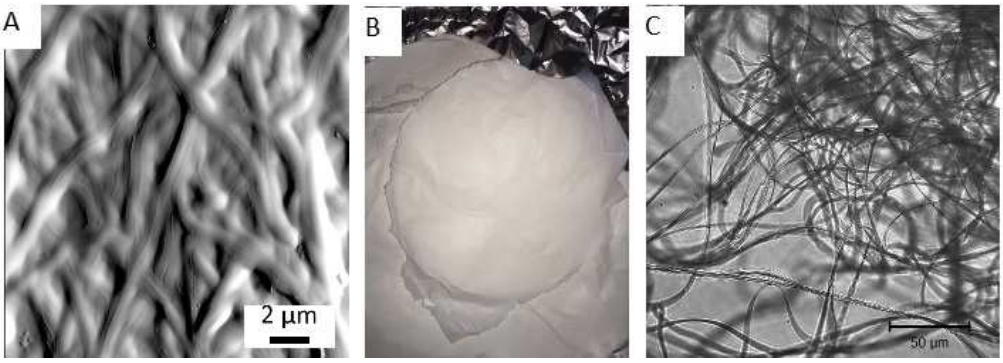

Figure 2: A) PVA Mesh Preparation, B) Macroscopical Picture of the PVA Mesh, C) Microscopical Picture of the PVA Mesh

In Vitro Analysis

For the direct contact assay a cell line of the Human lung epithelial carcinoma (A 549), was used. The cells were routinely maintained in a humidified incubator (5% CO_2, 37° C) with fresh medium changed in every second day. The cells were cultured with medium (DMEM, Sigma Aldrich, Budapest, Hungary) supplemented with 10% Foetal Bovine Serum (FCS, Sigma Aldrich, Budapest, Hungary), 2mM Glutamine (Sigma Aldrich, Budapest, Hungary) and Gentamicin (100 IU/mL and 100 g/mL, Sandoz). When the cells reached 70 % – 80 % confluence (2-4x10,000 cells/cm^2), they were suspended using 0.25% Trypsin (Sigma Aldrich, Budapest, Hungary). In group I., to determine the biocompatibility and toxicity of the solution of PVA was measured in different concentration ratio (10^{-8} to 0,8g/100 g). PVA solution were mixed together Glutaraldehyde (GDA) solution (0,001 and 0,1 M) as a crosslinker to avoid the polymer solubility in water. In group II., to study the adherence and morphology behaviour of the cells, hydrogel and scaffold formats were used. To create the hydrogel, we mixed together PVA solution (6 ml, 8 w %), 1M GDA (0.218 ml) and 3.91 ml distilled water. The samples were stored in PBS before the experiments. The PVA hydrogels (diameter: 25 mm, thickness: 1 mm) were placed in the Petri dish and the cells were seeded onto the top of the gels. The seeding was followed by 192 hours incubation. The photos were taken with inverted microscope (Olympus CK2). Finally, in group III., electrospun PVA scaffolds were placed with the cells in a 24-well plate (Sarstedt) and were incubated with the medium for 24hs, 48hs, 72hs, 96hs, 168hs (37 C, 5% CO_2). Control group contained A549 cells and medium without meshes. The electrospun PVA scaffolds washed with MQ water before cell experiments. Control cells were in the medium without nanomaterials. The cells were painted for the visualization with Trypan blue and the photos were taken with digital camera (DEM 130, Scope Photo software).

In Vivo Implantation of the Mesh

A total of fourteen adult male, Wistar rats, weighing between 200 and 250 g were used. The animals were kept under standard laboratory conditions and had free access to food and water. The animal experiment was approved by Hungarian National Food Chain Safety Office and followed the protocols approved by our Research Team. The animals were anesthetized with intramuscular Ketamin/Xylazine (Richter Gedeon Ltd., Budapest, Hungary) in 4:1 ratio. Thirty animals underwent mesh implantation, they were divided into 10 groups randomly. After left side transrectal laparotomy the tissue defect on the right side was covered with 2.5 cm piece of PVA mesh (n=30) and fixed with a 4/0 polypropylene (PP) suture (Figure 3A, 3B).

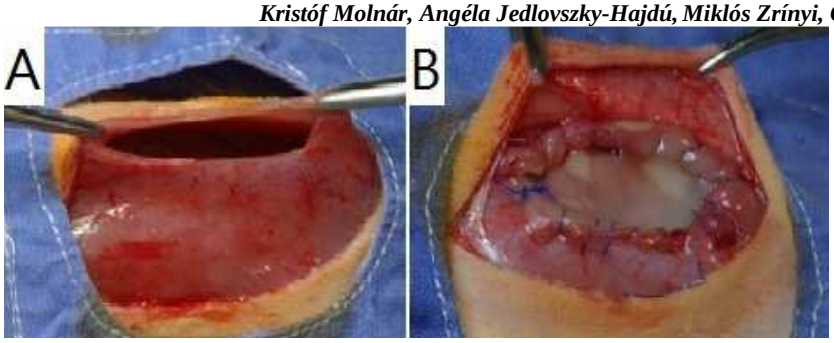

Figure 3: A) Abdominal Wall Defect, B) Implanted PVA Mesh

The skin was closed with simple interrupted suture line used 3/0 PP suture material. In the control animals (n=15), only a 4 cm long median laparotomy was performed than it was closed with simple interrupted suture line (3/0 PP). Reoperation was performed in the given postoperative days: 1st postop. day (POD) (n=3), 2nd POD (n=2), 3rd POD (n=2), 4th POD (n=2), 5th POD (n=2), 7th POD (n=3), 14th POD (n=1), 28th POD (n=2), 90th POD (n=3). Mesh adhesion were scored using a modified Diamond scale, where grade 0 meant 0 % adhesion, while grade 4 showed more than 75 % adhesions. According to the adhesion formation, three types were established: columnar (the adhered surfaces less than 0,5 x 0,5cm), curtain-like (the adhered surfaces 0,5cm < and 0,5 cm >) and large surface cohesive (the adhered surfaces more than 0,5cm x 0,5cm (Figure 4). The main purpose was to investigate the biocompatibility by measuring early and long-term signs of adhesion formation and inflammatory response through the connective tissue determination.

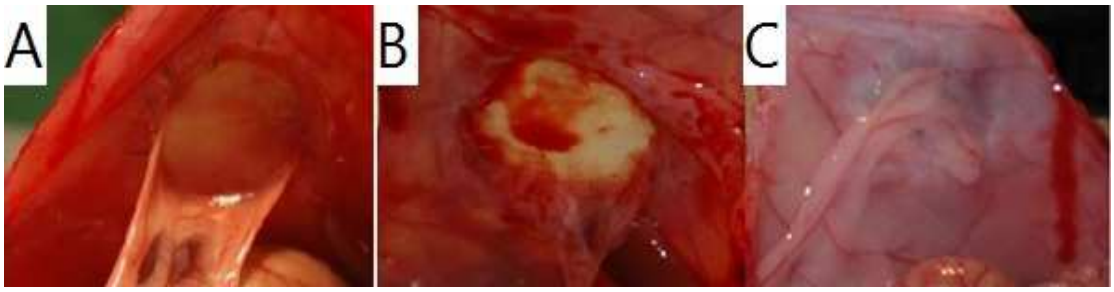

Figure 4: A) Curtain-Like Adhesion, B) Columnar Adhesion, C) Large Surface

Histological Evaluation

Tissue samples were fixed in 10% buffered formalin, embedded in paraffin and sectioned to 4 µm each and stained with Hematoxylin and Eosin. Glass slides were scanned with Pannoramic Scan (3DHISTECH, Budapest, Hungary) using Plan-Apochromat 20x magnification objective, a 1.6x camera adapter magnification and 1x Optovar magnification with a CIS VCC-FC60FR19CL camera, resulting in 0.24 µm/pixel resolution. Digital whole slides were evaluated using Pannoramic Viewer software (3DHISTECH, Budapest, Hungary). The inflammatory response against the mesh material was quantified according to the type and intesity of the reaction. The evaluation was done in a „blind" manner by two different pathologists.

RESULTS

Results of the in Vitro Experiments

After the 24 hours period the cells had normal, healthy shape in the solution, none of the cells were attached to the surface of the hydrogel. After 192 hours the cells were confluent in the Petri dish in the PVA solution, and they slowly formed to triangular shape with the hydrogel. (Figure 5A-B). Finally, in the last experiment we used 24 welled plates,

where the cells showed the normal morphology and could proliferated between the mesh and the bottom of the wells, on the other hand could not adhere to the surface of the mesh (Figure 5C-D).

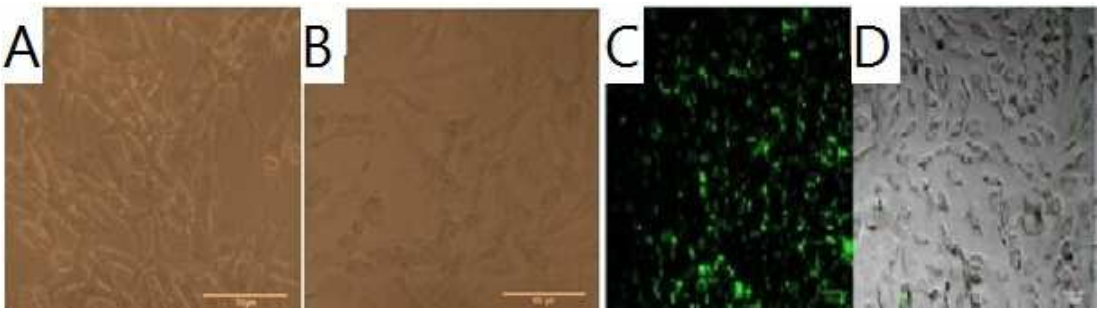

Figure 5: A-B) A549 Cells after 192 Hours Period on the PVA Hydrogel, C-D) A549 Cells on the PVA Scaffold after 192hs, Vizualization was evaluated with Vybrant Did

Macroscopical Findings of the in Vivo Animal Study

After the exploration of the abdominal wall the PVA mesh samples were evaluated macroscopically. Infection complications were not found in the environment of PVA meshes in none of the animals. In 5 animals some serous fluid could be detected between the PVA mesh and the skin. It was present only in the 1st (n=1), 2nd (n=2) and 3rd POD (n=3) and later could not be found anywhere. During the determination of the adhesion localization it was found that most of the adhesions were attatched to the non-absorbable suture material (n=19) and not to the surface of the PVA mesh (n=5). The adhesion attached to the mesh surface was less than 30 % of the total surface in all cases. Great omentum was the main tissue that took part in the adhesion formation in each animal. The proximity of the liver cause that this organ was mainly presence in the adhesions (n= 6).

In 6 cases the adhesion was instable especially in the early postoperative days (1-3 POD). Stronger traction needed for removing the moderate stable adhesion (n=13), which was dominant from the 3rd to 5th POD. In 8 animals stable adhesions could be found where the really strong pull could not remove the attached surfaces from each other at all. Adhesion formation was not typical in the control group. There were only two animals where columnar adhesion could be found. In both cases omentum attached to the incision line with a very fine surface.

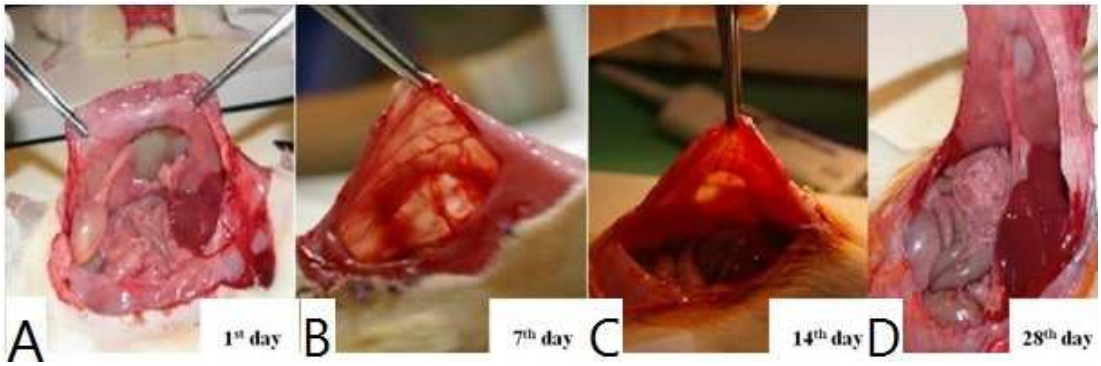

Figure 6: Implanted Mesh After, A) 1 Day, B) 7 Days, C) 14 Days and D) 28 Days

In groups which underwent implantation most of the adhesions were between 0 and 25 %. In six animals adhesions were between 25 and 50 %, and in three cases large adhesion formations were found on the surface of the mesh with other organs. Up to 75% adhesion formation were not found. The results based on the Diamond score were summarize in Table 1.

Table 1: Appearance of Adhesions in Animals According to the Diamond Score

Diamond Score	Extent of Adhesion (%)	Case (n)
0	0	1
1	1- 25	20
2	26-50	6
3	51-75	3
4	> 75	0

Histological Evaluation

There were likely a direct interaction between the PVA scaffold, as the extracellular matrix, and the tissue on both sides of the lesion. These structures create a scaffold that connects to the two faces of the lesion, allowing movement of cells into the scaffold. The PVA scaffold in our experiments created a permissive environment for growth while discouraging or preventing the scar formation which normally occurs at the early stage (Figure 7).

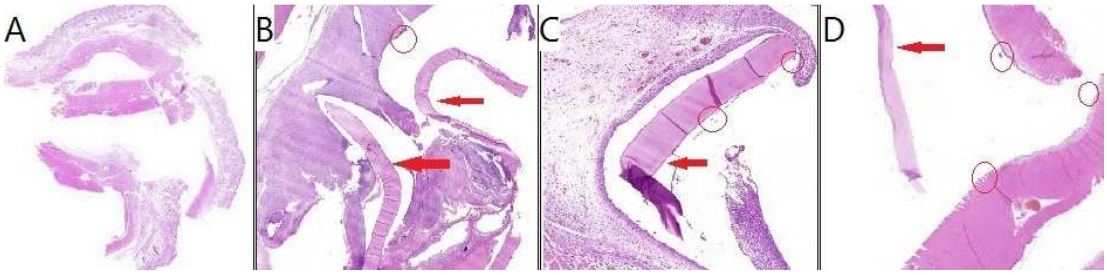

Figure 7: A) Control, B) Tissue Sample with the PVA Mesh after 7 POD (Red Arrows), C) Giant Cells on the Surface of the Mesh after 14 POD (Red Circles), D) PVA Mesh after the 28 POD without Post-Imflammatory Respons

CONCLUSIONS AND FUTURE WORK

Intraperitoneal positioning of the surgical mesh may cause adhesion formation. This study investigated the utility of a composite nanofiber Poly (vinyl alcohol) surgical mesh for the prevention of incisional hernia formation and evaluates the biomedical behaviour of the PVA hernia mesh after the implantation. Studies the surgical mesh implantation offer a basis for clinical transplantation. Various materials have been used; Vicryl (Ethicon), Prolene (Ethicon), 3D Max (BARD); however most of all are non-absorbable and have a lot of complication: adhesion formation, inflammatory response, mesh migration and chronic pain in the postoperative period after the surgery. There are only a limited number of publications that attempted to assess cell seeded 3D polymer scaffolds and 3D polymer scaffolds in animals.

In this preliminary research a novel 3D PVA scaffold was study in vitro and in vivo to characterize the biomedical properties. In vitro studies were focused on the toxicity of the fragments of the PVA. In vitro results show that the cells in the PVA solution and with the PVA hydrogel could proliferate. The PVA meshes and the degraded parts of the material did not cause any toxic effect for the cells. A549 cells have shown normal morphology, but the cells did not adhere to the surface of the mesh, it means the PVA scaffold is non-adhesive. Studies for the evaluation of the PVA mesh showed us, the cells had normal morphology in the petri-dish, but we still couldn't find too much cells on the surface of the samples. In our animal studies poly (vinyl alcohol) scaffolds were used to reconstruct the abdominal wall in rats. In this experimental animal model, we investigated the efficacy of the PVA scaffold on adhesion formation, and the inflammatory response. Several studies have described results about peritoneal adhesions. These adhesions are pathological bonds that typically

form between the omentum, the small and large bowels, the abdominal wall, and other intra-abdominal organs. These bonds may be a thin film of connective tissue, a thick fibrous bridge containing blood vessels and nerve tissue, or a direct adhesion between two organ surfaces [5]. Adhesions are the result of physiologic wound healing which develops on peritoneal surfaces due to inflammation, increases in vascular permeability and immune cells, and extravasation of fibrin deposits against trauma, ischemia, infection, and foreign bodies. Fibrinolytic activity usually hinders rapid adhesion formation; therefore, if fibrinolytic capacity is insufficient, adhesions develop. Adhesions are a severe postoperative complication and may develop even years after a surgical procedure; they are a cause of significant morbidity that may result in multiple complications. Depending on the etiology, peritoneal adhesions may be classified as congenital or acquired (post-inflammatory or post-operative) [6]. Some researchers assert that adhesions could also be classified in three major groups: adhesions formed at operative sites, adhesions formed de novo at non-operative sites, and adhesions formed after the lysis of previous adhesions [7]. 28 days of follow-up evaluation showed significantly higher the moderate adhesion format on the suture lines. It could be seen that the meshes integrated in the sorounding tissue. According to the Diamond score it was found that significantly more adhesion formations were attached to the suture line (n=19) than we could find on the surface of the mesh (n=5). Values were considered statistically significant for $P<0.05$. Diamond et al. distinguished types 1 and 2 of postoperative peritoneal adhesions. Type 1, or de novo adhesion formation, involves adhesions formed at sites that did not have previous adhesions, including Type 1A (no previous operative procedure at the site of adhesion) and Type 1B (previous operative procedures at the site of adhesion). Type 2 involves adhesion reformation, with two separate subtypes: Type 2A (no operative procedure other than adhesiolysis at the site of adhesion) and Type 2B (other operative procedures at the site of adhesions) [8]. Histological investigation of our samples showed all of the scaffolds were integrated to the host tissue and kept their structures until the end of the experiments. After the examination time, a solid connection was observed between the mesh and the abdominal wall. Histological response to PVA meshes was giant cell foreign body reaction in the early postoperative period, which was similar to surgical threads.

In summary, engineering tissues may provide an alternative to current therapies used to treat the loss or failure of tissue function. The estimated retention strength achivies with sutures in humans is much higer because the rat's abdominal muscle is about 5 mm thick, whereas that of humans is about 2 cm thick [9]. It also needs further examinations. This study demonstrated that the PVA nanofiber mesh is biocompatible with the cells and suitable for the long experiments in animals, it may provide an alternative option as biocompatible material for future treatment of abdominal wall defects.

ACKNOWLEDGEMENTS

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